



Photosynthetic response and proline bioaccumulation in black gram induced by Nickel stress

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ABSTRACT

The present study assess the impact of varying treatment concentrations of nickel (Ni) on photosynthetic responses and stress induced praline accumulation in a leguminous plant i.e. black gram [*Vigna mungo* (L.) Hepper] cultivars (PU31C and Shekhar1C). After exposure to different concentrations of toxic Ni²⁺, the two cultivars of *V. mungo* i.e. PU31C and Shekhar1C showed significant changes in their photosynthetic response. Sekhar1C variety showed high chlorophyll content in seedlings treated with toxic Ni (200µM) when compared to Control and other variety. PU31C variety showed four times more Chlorophyll content than seedlings of control treatment. The present hydroponic study exhibited stimulatory effects of nickel on total chlorophyll content, carotenoid and increased proline level after two weeks exposure period. Present preliminary study indicates the tolerance nature of two green gram cultivars to toxic doses of Ni²⁺.

Keywords: Nickel, Chlorophyll, Proline, Hydroponics

1. INTRODUCTION

Nickel holds a special place among the heavy metals. Unlike Cd, Pb, Hg, Ag, and several other metals that are not the components of plant enzymes, Ni is a constituent of urease, and small quantities of Ni (0.01 to 5 µg/g dry wt) are essential for some plant species. On the other hand, Ni is not as important for plant metabolism as Zn and Cu. However, same as with other heavy metals, high Ni concentrations may turn toxic to plants. The analysis of published evidence on Ni toxicity towards plants shows that, in addition to general toxicity displayed by all heavy metals, Ni manifests the specific characteristics due to its characteristic physical and chemical properties.

Nickel (Ni) occurs abundantly in igneous rocks as a free metal or as a complex with iron. It stands at twenty-second position amongst most abundant elements in the earth crust (Sunderman and Oskarsson 1991). Swedish chemist Ronstadt in 1751 discovered Ni with an atomic number 28 and an atomic weight of 58.71 but it exists in a number of oxidation states. Ni²⁺ form is stable over a wide range of pH and redox conditions prevailing in the soil. In general, naturally occurring concentration of Ni in soil and surface waters is lower than 100 and 0.005 ppm, respectively (McGrath 1995). Additionally, anthropogenic activities further release Ni into the soil through various sources such as smelting, burning of fossil fuel, vehicle emissions, disposal of house hold, municipal and industrial wastes, metal mining, fertilizer application, and organic manures (Alloway 1995).

Because of the competition between various metals in the course of their uptake by roots, some metals are absorbed in insufficient quantities, whereas the uptake of other metals is excessive. Such situation would indirectly predetermine the effects of heavy metals on the various facets of metabolism, such as photosynthesis, respiration, etc. It also seems important to compare the mechanisms of heavy metal accumulation, transport, toxicity, and detoxification in susceptible and tolerant plant species and populations. To this end, it was crucial to review the characteristic aspects of Ni²⁺ transport and distribution and Ni²⁺ effects on various physiological processes. In this aspect, Ni²⁺ is of particular interest due to numerous hyperaccumulators of nickel already discerned in various plant families.

The toxicity of Ni in plants has become a world-wide problem threatening sustainable agriculture as well. Ni, in contrast to other toxic trace (heavy) metals like cadmium, lead, mercury, copper and chromium has received little attention from plant scientists due to its dual character and complex electronic chemistry which is a major hurdle in disclosing its toxicity mechanism in plants. The critical toxicity level of Ni is more than 10 mg kg⁻¹ dry mass (DM) in sensitive species (Kozlow 2005), [50 mg kg⁻¹ DM in moderately tolerant species (Asher 1991) and [1,000 mg kg⁻¹ DM in Ni hyper accumulator plants such as Alyssum and Thalspi species (Kupper et al. 2001; Pollard et al. 2002). The impact of Ni toxicity on the physiology of plants depends on the type of plant species, growth stage, cultivation conditions, Ni concentration and exposure time (Krupa et al. 1993; Xylander and Braune 1994; Marschner 1995; Kabata-Pendias and Pendias 2001; Assuncao et al. 2003) in the soil. The toxic effects of higher concentration of Ni are observed at multiple levels, these include inhibition of mitotic activities (Rao and Sresty 2000), reduction in plant growth (Molas 2002), plant water relation and photosynthesis (Chen et al. 2009), inhibition of enzymatic activities as well as nitrogen metabolism (Gajewska et al. 2009), interference with the uptake of other essential metal ions (Chen et al. 2009), induction of oxidative stress (Chen et al. 2009). All of these alter physiological processes culminating ultimately in reduced fruit yield and quality (Gajewska et al. 2006).

In the laboratory condition several hydroponic studies have also been conducted to reveal growth responses of plants under adverse environmental conditions, which are considered critical for raising a successful agricultural crop. The present experimental works were performed to describe the effect of varying nickel concentrations on toxicological effects on photosynthesis and the changes on proline accumulation during the early stages of seedling growth in black gram (*Vigna mungo*) cultivars (Sekhar1C and PU31C).

2. MATERIALS AND METHODS

2.1. Plant Material

Dry seeds of black gram (*Vigna mungo* (L.) Hepper) cultivars (PU31C and Shekhar1) were collected from Orissa State Seed Corporation, Bhubaneswar. The seeds were stored in a bottle and kept in dark and cool place for experimental use.

2.2. Seedling growth

Germinated seeds were grown in hydroponics in controlled laboratory conditions in growth chambers. Well aerated hydroponic culture vessels containing Hoagland's nutrient solution (half strength) was treated as control and Hoagland's solution supplemented with different concentrations of Ni for seedling growth. The seedlings were grown under white fluorescent tubes (36 W Philips TLD) with a photon flux density of 52 µ/m²s (PAR) with a 12h photo period inside the growth chamber for 14 days.

2.3. Analysis of Chlorophyll Content

Chlorophyll analysis was conducted for 14 days old black gram seedlings grown in hydroponic culture experiments. The study was conducted by extracting chlorophyll in 80% acetone (Nadler *et al.*, 1972; Porra, 2002). The absorbance value of extracted liquid was recorded at 663.6 nm, 646.6 nm and 470 nm for Chlorophyll-a, Chlorophyll-b and carotenoid respectively.

2.4. Estimation of Proline content

Plant material (0.5) was grinded in 10ml of 3% sulfo-salicylic acid then the homogenized mixture was centrifuged at 3000 rpm for 10 minutes. Proline was estimated as per the method of Bates *et al.*, (1973). Then to the 2ml of supernatant 2ml of acid Ninhydrin reagent and 2ml of Glacial acid was added. This mixture was boiled in water bath at 100°C. The reaction was terminated, by placing the tubes in ice bath. 4ml of toluene was added to each of the test tube containing sample of different treatments. It was then allowed to separate into phases by vigorously using a cyclomixer. The chromophore containing upper toluene layer was collected carefully with help of micropipette and the absorbance was measured at 520nm.

3. RESULTS

Treatments of different Ni^{2+} concentrations (1 μM , 5 μM , 10 μM , 50 μM , 100 μM and 200 μM) showed marked changes in the different biochemical parameters of 14 days grown *Vigna mungo* (L.) Hepper seedlings. A comparative analysis of two cultivars of black gram i.e. Sekhar1C & PU31C were made in respect to their photosynthetic response and proline bioaccumulation induced by Ni. Comparative growth in respect to seedling height was clear from Plate 1 and Plate 2.



PLATE 1

COMPARATIVE GROWTH OF SEKHAR1C SEEDLINGS AFTER 14 DAYS EXPOSURE TO DIFFERENT CONCENTRATIONS OF NICKEL (SCREENING TEST) [From left to right: Control; Ni^{2+} (1 μM); Ni^{2+} (5 μM); Ni^{2+} (10 μM); Ni^{2+} (50 μM); Ni^{2+} (100 μM); Ni^{2+} (200 μM)]

Alterations in total chlorophyll content

Treatment of different Ni^{2+} concentrations (1 μM , 5 μM , 10 μM , 50 μM , 100 μM & 200 μM) along with control showed marked changes in the chlorophyll content of 14 days old *Vigna mungo* (L.) Hepper seedlings grown under Ni stress (Fig. 1). A marked increase in total chlorophyll content was observed in the seedlings treated with half strength Hoagland nutrient solutions. The total chlorophyll content decreased with increase in Ni^{2+} concentrations. Total chlorophyll content in the seedlings of Sekhar 1C variety & PU31C variety treated with Ni^{2+} (50 μM) & Ni^{2+} (10 μM) were found to be more than others. The order of total chlorophyll content treated with different nickel concentration was as follows-

In Sekhar 1C variety of black gram .:

Ni^{2+} (50 μM) > Ni^{2+} (1 μM) > Ni^{2+} (200 μM) > Ni^{2+} (100 μM) > Control > Ni^{2+} (10 μM) > Ni^{2+} (5 μM).

In PU 31C variety of black gram:

Ni^{2+} (10 μM) > Ni^{2+} (200 μM) > Ni^{2+} (100 μM) > Ni^{2+} (50 μM) > Ni^{2+} (1 μM) > Ni^{2+} (5 μM) > Control.

Sekhar1C variety showed better photosynthetic response than PU31C variety as reported from their chlorophyll level at different treatments of nickel.



PLATE 2

COMPARATIVE GROWTH OF PU 31C SEEDLINGS AFTER 14 DAYS EXPOSURE TO DIFFERENT CONCENTRATIONS OF NICKEL (SCREENING TEST) [From left to right: Control; Ni^{2+} (1 μM); Ni^{2+} (5 μM); Ni^{2+} (10 μM); Ni^{2+} (50 μM); Ni^{2+} (100 μM); Ni^{2+} (200 μM)]

Effect of Ni on Chlorophyll *a* content

A marked increase was found in chlorophyll *a* content of the seedling grown in controlled condition with increase in growth period. But with increased Ni^{2+} concentrations the chl-*a* content was decreased.

After 14 days treatment the decreasing trend of chlorophyll *a*. at different concentrations of nickel is as follows,

In Sekhar 1C variety of black gram

Ni^{2+} (50 μM) > Ni^{2+} (1 μM) > Ni^{2+} (200 μM) > Ni^{2+} (100 μM) > Control > Ni^{2+} (5 μM) > Ni^{2+} (10 μM).

In PU 31C variety of black gram :

Ni^{2+} (10 μM) > Ni^{2+} (200 μM) > Ni^{2+} (50 μM) > Ni^{2+} (100 μM) > Ni^{2+} (1 μM) > Ni^{2+} (5 μM) > Control.

Changes in Chlorophyll *b* content

There was a marked decrease in the chlorophyll *b* content in the seedlings. Chlorophyll *b*. content in the seedlings of Sekhar 1C variety & PU31C variety treated with Ni^{2+} (50 μM) & Ni^{2+} (200 μM) were found to be more than treated with others. The order of chlorophyll *b* content in the seedlings supplemented with Ni^{2+} solution was,

After 14 days treatment the decreasing trend of chlorophyll *b*. at different concentrations of nickel is as follows,

In Sekhar 1C variety of black gram :

Ni^{2+} (50 μM) > Ni^{2+} (1 μM) > Ni^{2+} (200 μM) > Ni^{2+} (100 μM) > Control > Ni^{2+} (5 μM) > Ni^{2+} (10 μM).

In PU 31C variety of black gram :

Ni^{2+} (200 μM) > Ni^{2+} (10 μM) > Ni^{2+} (100 μM) > Ni^{2+} (50 μM) > Ni^{2+} (1 μM) > Ni^{2+} (5 μM) > Control.

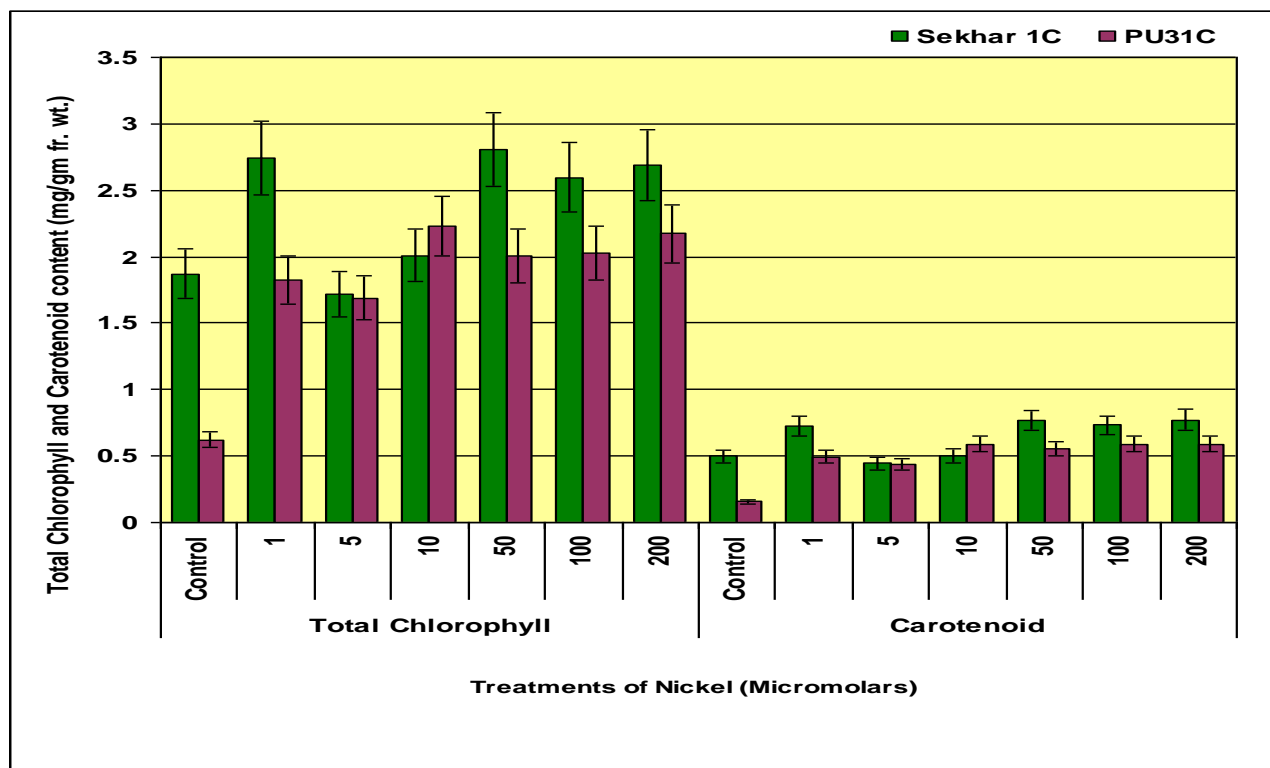


Figure 1

Bar graph with standard error bars showing a comparison of the effects of Nickel in two cultivated varieties (Sekhar1C and PU31C) of fourteen days grown *Vigna mungo* with respect to total chlorophyll and carotenoid content.

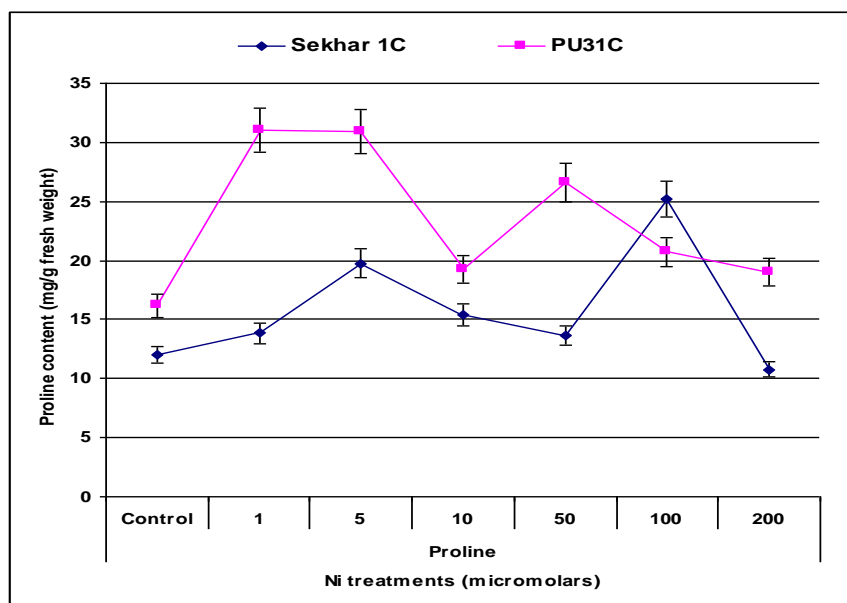


Figure 2

Effects of elevated supply of nickel on proline level in two cultivars of *Vigna mungo* seedlings treated for 14 days

Changes in Carotenoid content

After 14 days treatment the decreasing trend of Carotenoid at different concentrations of nickel is as follows,

In Sekhar 1C variety of black gram :

Ni^{2+} (200 μM) > Ni^{2+} (50 μM) > Ni^{2+} (100 μM) > Ni^{2+} (1 μM) > Control > Ni^{2+} (10 μM) > Ni^{2+} (5 μM).

In PU 31C variety of black gram:

Ni^{2+} (100 μM) > Ni^{2+} (200 μM) > Ni^{2+} (10 μM) > Ni^{2+} (50 μM) > Ni^{2+} (1 μM) > Ni^{2+} (5 μM) > Control.

The carotenoid content of Sekhar1C variety is better than PU31C variety.

Effect on proline content

Proline content in 14 days treated seedlings of *Vigna mungo* increased with increasing the dose of Ni in nutrient solution. It was found that maximum proline accumulation was observed in seedlings treated with Ni^{2+} (100 μM) in case of sekhar1C variety & Ni^{2+} (1 μM) in case of PU31C variety (Fig. 2).

After 14 days treatment the decreasing trend of proline at different concentrations of nickel is as follows,

In Sekhar 1C variety of black gram :

Ni^{2+} (100 μM) > Ni^{2+} (5 μM) > Ni^{2+} (10 μM) > Ni^{2+} (1 μM) > Ni^{2+} (50 μM) > Control > Ni^{2+} (200 μM).

In PU 31C variety of black gram :

Ni^{2+} (1 μM) > Ni^{2+} (5 μM) > Ni^{2+} (50 μM) > Ni^{2+} (100 μM) > Ni^{2+} (10 μM) > Ni^{2+} (200 μM) > Control.

The proline content of PU31C variety is better than Sekhar1C variety

4. DISCUSSION

In natural environment; the plants are exposed to various types of biotic and abiotic stresses. The phytotoxic effect of nickel was reported long time back. Uptake of nickel by plants results in reduced rate of growth, damage to cell wall, cell membranes and changes in the metabolic status of plants. In view of the seriousness of Ni pollution, the present study has been undertaken with an effort to assess the phytotoxic impacts with special reference to biochemical lesions in 14 days grown black gram seedlings. The part of the present hydroponics study provides a promising start for revising and comparing the level of Ni toxicity in two cultivars of black gram with a potentiality of their tolerance after exposure to varying concentrations of Ni. The work signifies the potential of black gram crop plants towards Ni phytotoxicity and tolerance.

Almost, at both the levels, Ni damages the photosynthetic apparatus/machinery, including the destruction of mesophyll cells and epidermal tissues (Bethkey and Drew 1992) and decreases the chlorophyll content (chlorophyll a, b, total chlorophyll and chlorophyll a/b ratio) (Gajewska et al. 2006; Ahmad et al. 2007; Alam et al. 2007; Gajewska and Sklodowska 2007). It also interferes with the photosynthetic electron transport chain (Mohanty et al. 1989) and the availability of its intermediates (such as cytochromes b6F and b559) in leaves (Krupa et al. 1993). The inhibition of electron transport is mainly on the donor side of photosystem II (PS II) (Singh et al. 1989) and the binding site for QB, the secondary quinone acceptor of PS II (Mohanty et al. 1989; El-Sheekh 1993).

In this paper, we investigated photosynthetic response of black gram under Ni stress condition. Typical symptoms Ni toxicity developed 15-17 days after the beginning of treatment. Both the varieties were found to be tolerant to Ni treatments even at high concentrations at an early stage of seedling growth. A comparison between two cultivars showed that Sekhar1C had five times more chlorophyll content than PU31C. A stimulatory effect of Ni was observed in both the varieties of seedlings at early stage of growth which may be attributed to increased synthesis of proline and enzyme activities. Our findings do not corroborate other researchers which may be due to the tolerance nature of these two varieties of Black gram.

Proline is an amino acid which could play a therapeutic role in plants. Proline, sugar, glycine, betaine and other organic solutes are believed to improve metal tolerance by contributing to osmosis and preserving enzyme activity in presence of toxic ions. Proline, an osmoprotectant, accumulate under heavy metal stress, and give rise to a series of reactions, which generate numerous free radicals by altered levels of major anions and accumulation. Proline is supposed to participate in the reconstruction of chlorophyll, activate the Krebs cycle and also in the energy source (Singh et al., 2012).

5. CONCLUSION

The above studies reveal the tolerance ability of two black gram cultivars towards Ni stress which could be implemented to screen them and cultivate them in contaminated soil. Intensive future research on the effects of accumulation of Ni and other heavy metal on plant metabolism is essential. Further the ability of other varieties for increasing crop yield and phytoaccumulation potential

needs to be tried. Suitable post harvest bioremediation techniques should be adopted for disposal of plants and plant parts containing accumulated Ni from the mining environment.

FUTURE ISSUES

These tolerant varieties of black gram seedlings should be grown in contaminated soil with *in situ* condition for getting better concrete result. Precise study on impact of Ni accumulation in plant metabolism is essential. Suitable post harvest bioremediation techniques should be adopted for disposal of plants and plant parts containing accumulated toxic Nickel from mining environment.

SUMMARY OF RESEARCH

1. Sekhar1C variety of black gram showed better growth response and photosynthetic response to elevated dose of Ni.
2. Proline accumulation was much elevated at 1 μM and 5 μM treatment of Ni.
3. A marked increase was found in carotenoid content of the seedling grown in Ni(200 μM) as compared to controlled condition in Sekhar 1C variety of *V. mungo*.
4. Total chlorophyll and carotenoid content in Sekhar1C variety of *V. Mungo* seedlings increased with increased treatment of Ni concentration.

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